

## AGGRESSIVENESS OF PRATYLENCHUS BRACHYURUS TO SUGARCANE, COMPARED WITH KEY NEMATODE P. ZEAЕ

Bruno Flávio Figueiredo Barbosa<sup>1\*</sup>, Jaime Maia dos Santos<sup>1</sup>, José Carlos Barbosa<sup>1</sup>, Pedro Luiz Martins Soares<sup>1</sup>, Anderson Robert Ruas<sup>2</sup>, Rafael Bernal de Carvalho<sup>1</sup>

<sup>1</sup>Jaboticabal Unit, UNESP São Paulo State University, Department of Plant Protection, Jaboticabal, SP, 14884-900, Brazil. <sup>2</sup>São Luiz College, Jaboticabal, SP, 14870-370, Brazil. The work is part of the doctorate thesis in Agronomy (Crop Production) of the first author. Author for correspondence: bruno.barbosa@posgrad.fcav.unesp.br

---

### ABSTRACT

Barbosa, B. F. F., J. M. dos Santos, J. C. Barbosa, P. L. M. Soares, A. R. Ruas, R. B. de Carvalho. 2013. Aggressiveness of *Pratylenchus brachyurus* to the sugarcane, compared with key nematode *P. zeaе*. *Nematropica* 43:119-130.

*Pratylenchus zeaе*, *Meloidogyne javanica* and *M. incognita* are considered key species of nematodes in sugarcane in Brazil, but *P. brachyurus* is also frequently found. This study was conducted to determine the aggressiveness of *P. brachyurus* compared with *P. zeaе* to sugarcane. Plants were grown in pots (100 L) in an open area with initial inoculation of 10, 100, 1,000, 10,000 and 100,000/plant for *P. brachyurus* and *P. zeaе*. The nematode inocula were from in vitro, carrot-cylinder cultures. Sampling was performed every 60 days until 300 days after inoculation. At harvest, we evaluated the population dynamics of the nematodes and plant growth characteristics. The population for the initial levels of 10 and 100,000 specimens/plant, for *P. brachyurus* and *P. zeaе* at 300 days after inoculation were similar. This fact shows that, upon detection of nematodes in a certain place during the planting of sugarcane, the ratoon on this area should be treated so as to control populations of *P. brachyurus* and/or *P. zeaе*. The damage caused by the initial population of 10 specimens of *P. brachyurus* was similar to those of 10,000 specimens of *P. zeaе*. The variety CTC 2 was classified as susceptible to *P. zeaе* and intolerant to *P. brachyurus*. Compared to the control, the losses as measured by the volume and fresh weight of shoots by the nematode species were 29.82% and 40.34%, respectively. *Pratylenchus brachyurus* was more aggressive than *P. zeaе* to the CTC 2 sugarcane variety.

*Key words:* Root lesion nematodes, resistance, *Saccharum* spp., host-parasitic relationship, plant disease loss.

---

### RESUMO

Barbosa, B. F. F., J. M. dos Santos, J. C. Barbosa, P. L. M. Soares, A. R. Ruas, R. B. de Carvalho. 2013. Agressividade de *P. brachyurus* à cana-de-açúcar, comparada ao do nematoide-chave *P. zeaе*. *Nematropica* 43:119-130.

*Pratylenchus zeaе*, *Meloidogyne javanica* e *M. incognita* são consideradas espécies-chave de nematoides na cana-de-açúcar no Brasil, porém, *P. brachyurus* é encontrada em amostras provenientes de canaviais de várias localidades. Esse trabalho foi conduzido para averiguar a agressividade de *P. brachyurus* comparada a de *P. zeaе* à cana-de-açúcar. Foram utilizados vasos (100 L), a céu aberto, com os níveis de inóculo inicial 10, 100, 1.000, 10.000 e 100.000 espécimes/planta, para os dois nematoides, isoladamente, em terra tratada com vapor de caldeira agrícola. O inóculo dos nematoides foi obtido in vitro, em cilindros de cenoura. Foi realizada amostragem prévia e, a cada 60, até 300 dias após as inoculações. Por ocasião do corte, foram realizadas avaliações nematológicas e de caracteres relacionados ao desenvolvimento da cultura. A população para os níveis iniciais de 10 e 100.000 espécimes/planta, tanto para *P. brachyurus* aos 300 dias quanto *P. zeaе* aos 240 e 300 dias após a inoculação, já se assemelhava, portanto, após o 1º corte, já seria necessário o controle desses nematoides na soqueira. Os danos causados por 10 espécimes de população inicial de *P. brachyurus* foram semelhantes à de 10.000 espécimes de *P. zeaе*. A variedade CTC 2 foi classificada como suscetível à *P. zeaе* e intolerante à *P. brachyurus*. As perdas provocadas ao volume estimado e à massa fresca da parte aérea, pelas espécies de nematoide, considerando-se todos os níveis inoculados, foram respectivamente de 29,82% e 40,34%. *Pratylenchus brachyurus* foi mais agressivo que *P. zeaе* à variedade de cana-de-açúcar CTC 2.

*Palabras clave:* Nematode das lesões radiculares, resistência, *Saccharum* spp., inter-relações patógeno-hospedeiro, danos causados por doenças.

## INTRODUCTION

*Pratylenchus zae* Graham, *Meloidogyne javanica* (Treub) Chitwood and *M. incognita* (Kofoid and White) Chitwood are considered the key damaging species of nematodes in sugarcane culture in Brazil (Cadet and Spaul, 2005; Dinardo-Miranda, 2005). These species are widely distributed in major producing regions of the country and cause severe damage to roots and yield of sugarcane.

The longevity of sugarcane (*Saccharum* spp.) favors multiplication of these species of nematodes. Moreover, crop damage by nematodes increases in warmer years with regular rainfall. When grown as a monoculture, replanting sugarcane often occurs without a fallow period between removal of old stumps and replanting. These conditions favor development of increasing nematode populations (Spaul and Cadet, 1990).

The *Pratylenchus* species causes necrosis of the roots, attacking primarily the fibrous root, thus reducing the absorption of water and nutrients by the plant. Poor root development is reflected in poor development of the aerial part of the plant, causing severe damage and resulting in reduced crop yield. In the field, visual symptoms are manifested in the form of "patches" or "foci" of stunted plants with chlorosis and varying degrees of nutritional deficiency, as well as dry leaf-edges, in contrast with surrounding plants that do not have visual symptoms of nutrient deficiency (Cadet and Spaul, 2005). However, final confirmation of the cause of any field damage requires sampling of the roots and soil, and laboratory analysis.

For *P. zae*, field tests showed that the application of nematicides at planting of several varieties contributed to highly significant increases in productivity, reaching values of up to 40,000 Kg.ha<sup>-1</sup> (Dinardo-Miranda *et al.*, 1998). Study of the damage caused by *P. brachyurus* and *P. zae* to SP70-1143 and SP71-1406 varieties of sugarcane was conducted by Dinardo-Miranda (1990). The author found that *P. brachyurus* multiplied in roots of both varieties, with no harm to either of them, and considered both varieties tolerant to the nematode. *Pratylenchus zae* caused no reduction in productivity in the variety SP70-1143, which was characterized as resistant. However, for variety SP71-1406, *P. zae* caused significant losses in productivity, and the variety was considered susceptible. In contrast, Dinardo-Miranda (2005) cites the species *P. brachyurus* as very common in sugarcane plantations in São Paulo, but the aggressiveness of this species to sugarcane has not been adequately evaluated.

*Pratylenchus brachyurus* has been consistently found in samples sent to the Nematology Laboratory FCAV/UNESP, Jaboticabal, from different sugarcane producing regions in Brazil. Given the reported significant influence of *M. incognita*, *M. javanica* and *P. zae* on sugarcane yield, work was initiated to ascertain whether *P. brachyurus* also significantly

influences the productivity of sugarcane compared with *P. zae*.

## MATERIALS AND METHODS

### *Experimental conditions and treatments*

This experiment was carried out in an open area of the São Martinho Plant, Pradópolis, SP. Each experimental unit was represented by seven individual plants in pots (60 cm dia x 50 cm high) containing 100 L of podzolic soil that had been steam-heated to 80°C for 2 hours to kill pathogens.

The experiment was a randomized block design with a factorial arrangement of treatments: two nematode species (*P. zae* and *P. brachyurus*) each with six inoculum levels (no nematodes and 10, 100, 1,000, 10,000, and 100,000 vermiform and eggs/plant), each block with five replicates. Therefore, 5 blocks with 11 experimental units per block totaled 55 plots with seven pots by plot. The plots were spaced at 1 m apart with 4 m spacing between blocks.

### *Planting and conduction of the experiment*

Sugarcane variety CTC2 was planted, three stalks per pot with a viable germ in each stalk (09.10.2007). All plants were watered daily as needed. After sprouting (10.20.2007), the plants were thinned to one plant per pot.

### *Inoculum preparation and inoculation*

The subpopulation of *P. brachyurus* inoculum came from Santa Adélia, SP and *P. zae* from Onda Verde, SP. These two subpopulations were extracted from sugarcane roots and multiplied *in vitro* in the Nematology Laboratory FCAV / UNESP, in carrot cylinder culture (Gonzaga *et al.*, 2008).

At 120 days after inoculation, the nematodes were extracted from the carrot cylinders, using Coolen and D'Herde's (1972) technique. A Peter's counting chamber was used to adjust the inoculum for each treatment. On 10.20.2007, the root system of the test plants was partially exposed, inoculated with a suspension of 100 mL for each of the nematode species separately per plant, and the roots immediately covered with the potting soil

### *Evaluation of the experiment*

To quantify populations of each *Pratylenchus* spp., periodic samplings were taken every 60 days until 300 days (10.20.2007 to 08.15.2008) after inoculation. A sample consisted of three subsamples, one per pot, of soil and roots from each plot, including in the control. These plants were not sampled in the immediately subsequent evaluation, thus minimizing the stress caused to them by the sampling. The samples were

stored in plastic bags, and transported for processing at the Nematology Laboratory, Department of Plant Protection, UNESP / FCAV, Jaboticabal. Nematodes were extracted from 100 cm<sup>3</sup> of soil by centrifugal flotation (Jenkins, 1964) and from 10 g of roots using sucrose solution (45,4%) (Coolen and D'Herde, 1972). The identification of the species present in each sample was confirmed with morphology and morphological measurements described in Castillo and Vovlas, 2007, and the population was estimated using a Peter's chamber (Southey, 1970). At harvest (10.20.2008), data collected were: number of nematodes per 100 cm<sup>3</sup> of soil and per 10 g of roots, fresh weight of shoots and tips, number of tillers, diameter and length of stalks (Landell and Silva, 1995), estimated volume of stalks (circular area x length of stalks), number of stalks suitable for industrialization, number of internodes of the stalks and analysis of technological variables [Percentage of the apparent mass of sucrose (PC), Purity or percentage of sucrose in relation to total soluble solids of the juice extracted from stalks, Total reducing sugars (TRS) and Percentage of total soluble solids (Brix%) of juice extracted from stalks]. The tip mass was obtained from the break at the last sprouting. Data were subjected to statistical procedures, using regression analysis. The choice of model was based on the coefficient of determination, the significance of the regression coefficients, tested by F test, considering an acceptable level of up to 5% probability. Nematode frequency data were transformed [ $\log(x + 5)$ ] prior to analysis and all data was analyzed using the Agroestat (2011) program. Possible interrelationships between nematode population levels and the different variables were considered.

For preparation of systematic discussion of compared aggression of *P. brachyurus* and *P. zae* the following criteria were used: Evaluation of the effect of inoculation with nematodes at increasing levels on population change over time for each species evaluated in isolation, and evaluation of the compared effect between the species of nematodes on plant biometric and quantitative variables and fresh weight.

## RESULTS AND DISCUSSION

### *Effect of inoculation in increasing levels of Pratylenchus brachyurus and P. zae on the population dynamics of these species of nematode*

The small proportion of nematodes in the soil in relation to the roots caused low variation in final populations in soil among the initial inoculum. However, reproductive factors and climate influenced the differences between the evaluation periods, both in soil and roots (Table 1).

The population density of *P. brachyurus* in the soil, averaged across all inoculum levels, increased proportionally to the increase in population level of the original inoculation. The number of specimens,

*Pratylenchus* spp. in the soil when the host crop is growing in the field, is usually lower than in the roots. This fact is related to the migratory behavior of endoparasitic nematodes. When the plant senesces and roots die, these nematodes migrate to the closest healthy root (Castillo and Vovlas, 2007). However, there is the seasonality of the population over time of evaluation, due to climatic influence. The population peak of *P. brachyurus* in soil was between 150 and 160 days (March 2008) after inoculation of the nematodes. From the inflection point of the curve onwards, the values fell progressively in accordance with how high the initial levels of inocula were. Castillo and Vovlas (2007) observed that the population of *P. brachyurus* in soil under maize cultivation exhibited fluctuation with a decline during the growth period, probably caused by the migration of nematodes from the soil to the roots.

The inoculum levels did not influence the change in the population of nematodes present in the soil at 60, 240 and 300 days after inoculation. However, after 180 days, there was a linear increase in the number of specimens directly proportional to the level of initial inoculum. This did not occur in the assessment at 120 days where there was an increase in the number of nematodes up to the level of initial inoculum of 1,000 specimens/plant. Beyond the 1,000 specimen level, the curve follows a trend of increasing population in the period until, at an intermediate point between the 1,000 and 10,000 levels, there is inflection of the curve, initiating a gradual reduction in the population of nematodes in the period up to the initial inoculum level of 100,000 specimens/plant.

The differences between the levels of inoculum in relation to the roots is justified because, of the endoparasitic migratory behavior, in the presence of living roots the number of *Pratylenchus* spp. is considerably higher in the roots. There was a gradual increase in the population of *P. brachyurus* in soil until the 1,000 specimen level and a reduction when available space and food in the roots become a limiting factor (inoculum levels 10,000 and 100,000), due to self-competition (Almeida, 2011). At 180 days, root growth provided vital space sufficient to support progressive increases in population directly proportional to the level of initial inoculum. Subsequent evaluations revealed a reduction in the general population, and the level of initial inoculum no longer influenced the number of specimens present in the soil (Fig. 1A, B).

The number of individuals found in the roots increased linearly in direct proportion to the increased level of initial inoculum. Although there had been a population reduction at the 100,000 individuals/plant level at 120 days, probably the nematodes that survived in the soil migrated to the roots that were formed at the time, resulting in increased population density of nematodes in the roots at 240 days, highlighting this inoculum treatment as the most favorable to the multiplication of *P. brachyurus* in the study period (Fig. 2A). Considering all levels of

Table 1. Analysis of variance for the population of *Pratylenchus brachyurus* and *P. zaeae*, inoculated with increasing levels in 2 CTC variety sugarcane (20/10/2007), evaluated after five periods of 60 days (Pradópolis, SP).

Population density (P)	<i>Pratylenchus brachyurus</i>		<i>P. zaeae</i>	
	NNL <sup>x</sup>	NNR <sup>y</sup>	NNL	NNR
10/plant	1.4	44.3	60.2	831.4
100/plant	4.8	68.7	53.1	358.8
1,000/plant	9.7	208.5	83.6	910.0
10,000/plant	17.6	394.8	99.0	1152.9
100,000/plant	12.9	263.5	91.4	766.6
F Test for P	2.4 <sup>NS</sup>	3.2*	0.9 <sup>NS</sup>	2.2 <sup>NS</sup>
Evaluation Periods (EP) <sup>z</sup>				
60	0.3	2.5	16.8	305.4
120	4.3	345.1	166.8	2030.2
180	21.9	286.6	140.5	1043.1
240	5.8	207.0	38.2	272.8
300	4.1	138.7	25.1	368.0
F Test for EP	7.86**	2.5**	19.5**	18.1**
F Test for P x EP	0.94 <sup>NS</sup>	0.9 <sup>NS</sup>	0.6 <sup>NS</sup>	1.6 <sup>NS</sup>
C.V. (%)	42.0	69.1	25.3	41.4

NNL<sup>x</sup> = number of nematodes in 100 cm<sup>3</sup> of soil; NNR<sup>y</sup> = number of nematodes in 10 g of roots; \* Significant difference at 5% and \*\* at 1% of probability; NS No significant differences; (EP)<sup>z</sup> = Evaluation Periods in days after inoculation

Table 2. Analysis of variance for number of tillers, internodes, and stalks suitable for industrialization in sugarcane plants, variety CTC 2, inoculated with increasing levels of *Pratylenchus brachyurus* or *P. zaeae* and without inoculation. Pradópolis, October 20, 2008.

Nematode (N)	Tiller number per plant	Internode number per stalk	Number of stalks suitable for industrialization per plant
Pb	9.07	22.0	4.7
Pz	9.03	21.5	4.4
F Test for N	0.00	1.72	1.80
Nematode Population density(P)			
10/plant	8.8	21.8	4.2
100/plant	8.2	22.0	4.5
1,000/plant	9.5	21.7	4.4
10,000/plant	9.8	21.7	5.0
100,000/plant	8.9	21.6	4.6
F Test for P	0.66	0.10	0.92
F Test for N x P	0.23	2.65*	1.17
Control (C)	7.0 a	23.0 a	6.3 a
Factorial (F)	9.0 a	21.8 a	4.5 b
C vs. F	3.13	3.44	14.67**
C.V. (%)	27.83	6.47	21.19

\* Significant difference at 5% and \*\* at 1% of probability; Pb = *Pratylenchus brachyurus* and Pz = *Pratylenchus zaeae*.

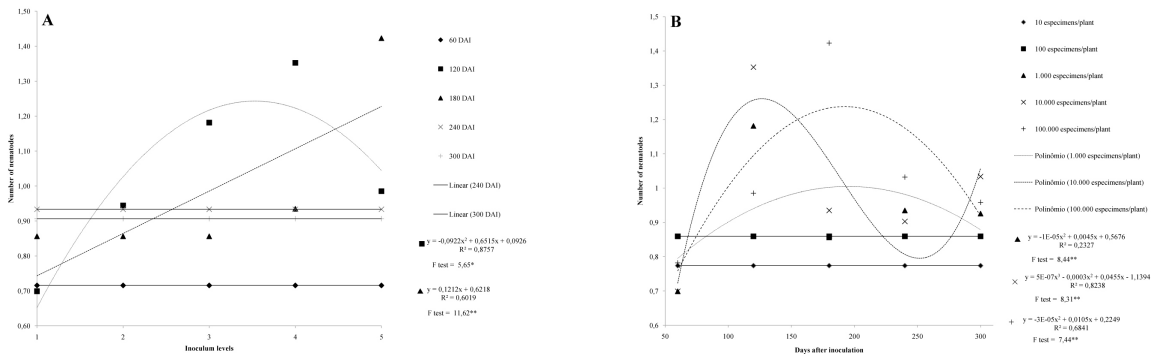


Fig. 1. Inter-relationship between initial inoculum and population of *Pratylenchus brachyurus* in soil planted with sugarcane, CTC 2 variety, evaluated after five periods of 60 days. A) Effect of increasing levels of initial inoculum over five consecutive time periods. The X axis with initial inoculum transformed into  $\log$  B) Fluctuation over the course of a year for different levels of inoculum. Averages transformed into  $\log (x + 5)$  for regression analysis.

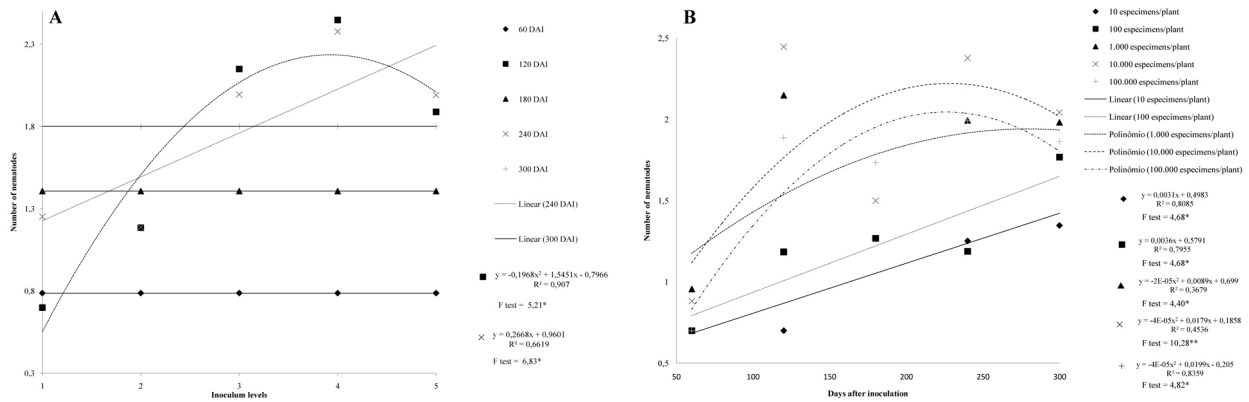


Fig. 2. Inter-relationship between initial inoculum and population of *Pratylenchus brachyurus* in roots of sugarcane, CTC 2 variety, evaluated after five periods of 60 days. A) Effect of increasing levels of initial inoculum over five consecutive time periods. The X axis with initial inoculum transformed into  $\log$  B) Fluctuation over the course of a year for different levels of inoculum. Averages transformed into  $\log (x + 5)$  for regression analysis.

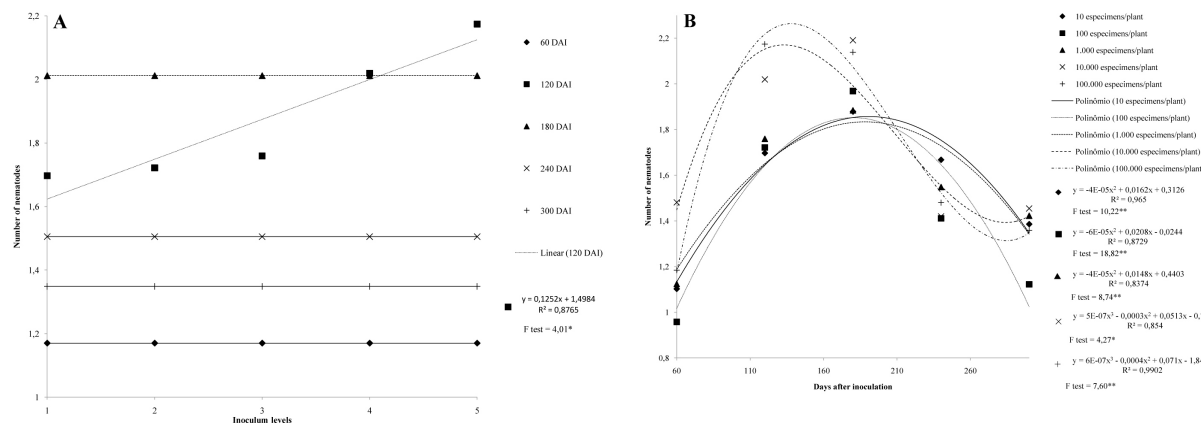


Fig. 3. Inter-relationship between initial inoculum and population of *Pratylenchus zae* on soil planted with sugarcane, CTC 2 variety, evaluated after five periods of 60 days. A) Effect of increasing levels of initial inoculum over five consecutive time periods. The X axis with initial inoculum transformed into  $\log$  B) Fluctuation over the course of a year for different levels of inoculum. Averages transformed into  $\log (x + 5)$  for regression analysis.

initial inoculum of *P. brachyurus*, the population peak occurred approximately 170-180 days (April 2008) after inoculum application.

The data of Figure 2B show that with initial inoculation of 10 and 100 specimens of *P. brachyurus*/plant, due to low initial infestation, the values for the population in the different periods were among the lowest compared to other levels. However, the populations increased linearly over time. For plants that received 1,000, 10,000 and 100,000 individuals/plant, the population peak was similar, with the density of 10,000 individuals/plant standing out from the others as having a greater number of nematodes in all periods.

Across the five assessment periods for *P. zaeae*, the level of initial inoculum did not significantly influence the population found in the soil. However, assessment period influenced the populations in both roots and soil. The populations of *P. zaeae* peaked much earlier than populations of *P. brachyurus* (Fig. 4). This fact is most likely related to greater biotic potential of *P. zaeae*

compared with *P. brachyurus*, considering the average population values for both species (Table 1). Olowe and Corbet (1976) cited by Kimenju *et al.* (1998) attribute the field population dominance of *P. zaeae* over *P. brachyurus* to its greater biotic potential and greater tolerance to environmental stress factors.

Figure 4A shows a similar trend in *P. zaeae* population values in roots from the five levels of inoculum, except for the values obtained at 240 and 300 days, where level was not a significant influence, showing population equality among the treatments related to *P. zaeae*. Figure 4B represents the fluctuation of *P. zaeae* population during the five assessments. The inflection of the curves was very similar between the different levels of initial inoculum, occurring between 130 and 140 days after treatment application. The population peak of *P. zaeae* in the roots was earlier than the peak for *P. brachyurus* (Fig. 2B) indicating a greater reproductive capacity of *P. zaeae* (Fig. 4B). Compared to the other population levels, 100 specimens of *P. zaeae* only peaked 210 days after inoculation, similar

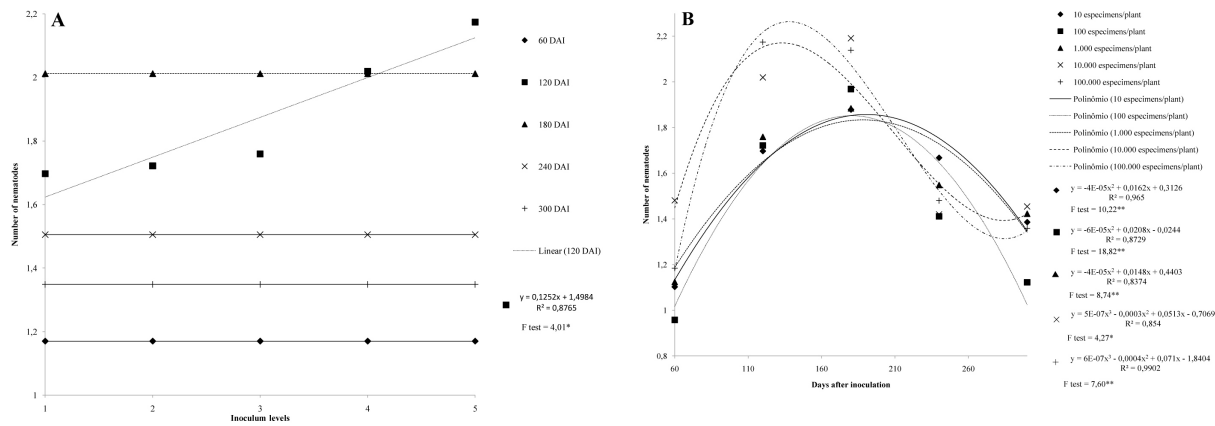


Fig. 3. Inter-relationship between initial inoculum and population of *Pratylenchus zaeae* on soil planted with sugarcane, CTC 2 variety, evaluated after five periods of 60 days. A) Effect of increasing levels of initial inoculum over five consecutive time periods. The X axis with initial inoculum transformed into  $\log$  B) Fluctuation over the course of a year for different levels of inoculum. Averages transformed into  $\log (x + 5)$  for regression analysis.

Table 3. Unfolding of the interaction between *Pratylenchus brachyurus* and *P. zaeae* in their respective initial inoculums for the number of internodes present. Pradópolis, October 20, 2008.

Species of nematode	Inoculum levels					F Test
	10	100	1000	10.000	100.000	
Pb	21.8 Aa	21.6 Aa	21.4 Aa	22.4 Aa	22.9 Aa	0.98
Pz	21.9 Aa	22.3 Aa	22.1 Aa	20.9 Aa	20.3 Ab	1.77
F Test	0.01	0.6	0.6	2.7	8.4**	-

\*\* Significant difference at 1% probability to the F test; Pb = *Pratylenchus brachyurus* and Pz = *Pratylenchus zaeae*. Averages followed by same uppercase letters in the row and same lowercase letters in the column do not differ statistically from one another for the Tukey test at 5% of probability.

Table 4. Analysis of variance for stalk diameter at the third internode, Length of stalk until insertion leaf +1, and estimated stalk volume in plants of the CTC 2 variety, inoculated with increasing levels of *Pratylenchus brachyurus* or *P. zaei* and without inoculation. Pradópolis, October 20, 2008.

Nematode (N)	Volume (dm <sup>3</sup> )	Diameter of stalk (mm)	Length of stalk until insertion leaf +1 (m)	Fresh weight of the stalks (Kg/plant)
Pb	0.38	21.9	2.2	1.68
Pz	0.41	22.7	2.2	1.84
F Test for N	1.21	1.29	0.23	0.93
Population (P)				
10/plant	0.42	23.1	2.2	1.68
100/plant	0.41	22.6	2.3	1.82
1,000/plant	0.41	22.5	2.2	1.66
10,000/plant	0.37	21.4	2.1	1.70
100,000/plant	0.39	22.1	2.3	1.96
F Test for P	0.52	0.61	1.80	0.47
F Test for N x P	1.39	1.33	1.28	1.07
Control (C)	0.57 a	27.0 a	2.7 a	2.95 a
Factorial (F)	0.40 b	22.3 b	2.2 b	1.76 b
F Test for C vs. F	17.76**	15.56**	20.45**	18.36**
C.V. (%)	21.40	11.07	10.40	31.45

\*\* Significant difference at 1% probability to the F test; Pb = *Pratylenchus brachyurus* and Pz = *Pratylenchus zaei*.

Table 5. Analysis of variance for technological variables [Percentage of the apparent mass of sucrose (PC), Purity or percentage of sucrose in relation to total soluble solids of the juice extracted from stalks, Total reducing sugars (TRS) and Percentage of total soluble solids (Brix%) of juice extracted from stalks] for the stalks of plants of the variety CTC 2, inoculated with increasing levels of *Pratylenchus brachyurus* or *P. zaei* and without inoculation. Pradópolis, October 20, 2008.

Nematode (N)	Brix(%)	Pol% cane (PC)	Purity	Fiber (%)	TRS
Pb	21.32	15.7	89.72	13.87	153.51
Pz	21.14	15.6	89.45	13.59	152.64
F Teste for N	0.86	0.38	1.64	1.71	0.33
Population (P)					
10/plant	21.21	15.57	89.72	13.96	152.5
100/plant	20.74	15.25	89.24	13.57	149.6
1,000/plant	21.41	15.82	89.76	13.61	154.9
10,000/plant	21.40	15.73	89.72	13.90	154.0
100,000/plant	21.38	15.76	89.48	13.60	154.4
F Teste for P	1.75	1.59	0.86	0.58	1.60
F Teste for N x P	4.26*	4.88**	5.21**	1.04	4.78**
Control	21.43	15.81	89.74	13.72	154.8
Factorial	21.23	15.62	89.58	13.73	153.1
F Teste for C vs. F	0.40	0.48	0.19	0.00	0.48
C.V. (%)	2.50	2.85	0.65	4.34	2.70

\*\* Significant difference at 1% probability to the F test; Pb = *Pratylenchus brachyurus* and Pz = *Pratylenchus zaei*.

to results obtained for levels greater than or equal to 1,000 specimens of *P. brachyurus*. This fact denotes the low plant capacity to support high populations of *P. brachyurus*.

The data on population of the two nematode species indicate that the variety of sugarcane CTC 2 can be classified as susceptible to *P. zaeae* and intolerant to *P. brachyurus*. Susceptibility is the plant's capacity to support the growth and reproduction of nematodes (Hartman *et al.*, 1985). The terms tolerance/intolerance are used to describe the ability of the plant to withstand nematode infection; intolerant plants suffer injuries, present less growth and may even die (Evans and Haydock, 1990; Trudgill, 1991).

*Effect of inoculation with increasing levels of Pratylenchus brachyurus and P. zaeae on biometric and quantitative variables*

The initial inoculum of *P. brachyurus* and *P. zaeae* did not influence the number of tillers and stalks suitable for industrialization. However, the number of suitable stalks was significantly higher in the control (Table 2). There were higher internode numbers with *P. brachyurus* than with *P. zaeae* at the 100,000 specimens/plant level (Table 3).

The nematodes species, their levels of inoculum and the control did not differ statistically in relation to fresh weight of the tips. However, significantly higher values of fresh weight of the aerial parts were obtained for the control in relation to the other treatments, which showed no significant difference between them. The volume and fresh weight for control was 0.57 dm<sup>3</sup> and 2.95 kg/plant, however, for the factorial plants they were 0.40 and 1.76 kg dm<sup>3</sup>/plant, respectively, so losses caused by both species of nematodes, considering all levels inoculated were 29.82% and 40.34% (Table 4).

There were no significant differences between the nematode species and their respective levels in relation to the variables: stalk Volume and Diameter and stalk Length to the first leaf (leaf +1). However, the no nematode control stood out with the highest values for all three variables (Table 4), showing the damage to crops caused by nematodes. The levels of *P. zaeae* influenced these variables in a manner inversely proportional to the increase in values (Fig. 5). In other words, the higher the population level, the lower the value of the variables, except the length of the leaf +1, which presented a small increase between the initial inocula of 10 and 100 specimens/plant. From this level onwards, it reduced the variable values up to the 10,000 level, where there is a new inflection of the curve. The oscillations occurring in the length to the leaf +1 on the plants for *P. zaeae* must be related to two inter-relationships between pathogen and host. The first is the response of the plant after the breakdown of apical dominance of the roots caused by the attack of the pest at low population levels (10 and 100 specimens/plant); the plant tries to recover by issuing lateral

roots that end up stimulating the early development of the plant. With the increase of infestation, the plant cannot compensate for the damage and begins to show retardation in its development. Secondly, in the increase from the 10,000 level, the high initial infestation level provided by the 100,000 nematodes/plant, caused fierce competition among the specimens of *P. zaeae* for roots. Consequently, there was greater root death and starvation of many of the inoculated nematodes, resulting in reduction of infestation in the first 60 days. With its constant renewal of roots, the sugarcane was able to recover a little, but due to high infestation of the soil by surviving individuals, the damage continued to occur as can be seen when analyzing the other biometric data (Fig. 5).

Almeida *et al.* (2011) studied the effect of increasing levels of *M. enterolobii* (Young and Eisenback, 1983) in guava and found that the highest level used (10,000 eggs and J2/plant) provided competition between the specimens and reduced the population of nematodes. The values of estimated volume and diameter of the stalks of plants inoculated with *P. brachyurus* in their respective levels were similar to those obtained for the level of 10,000 specimens of initial inoculum of *P. zaeae* (Fig. 5). Accordingly, 10 individuals present initially were sufficient to cause damage similar to that caused by 10,000 *P. zaeae* individuals. The number of stalks suitable for industrialization was significantly higher for the control, showing the damage to crops caused by these species of nematode.

An increased number of internodes in a given length results in more fiber and less sucrose. Therefore, as there were a greater number of internodes on the stalks of plants inoculated with *P. brachyurus* and there were no significant differences in the average length of the stalks for the species of nematodes (Table 4), *P. brachyurus* caused greater damage to the crop.

Barbosa (2009) evaluated nine varieties of sugarcane for the reproduction rate of *P. brachyurus* (final population/initial population) to assess resistance at ninety days (reproduction factor less than 1). Only the SP801816 variety was classified as susceptible, and CTC 2 was classified as resistant. Considering these results and those obtained in the present research, we can infer that the low reproductive rate of *P. brachyurus* in relation to *P. zaeae* in sugarcane varieties hampers the detection of *P. brachyurus* at high populations in the field, however, these low populations can result in more damage than high populations of *P. zaeae* when the varieties are hypersensitive to *P. brachyurus*. The simple presence of *P. brachyurus* in samples taken immediately after cutting or in areas of reform may be enough to cause damage to the crop.

Fiber% did not differ with respect to nematode species. The other variables showed differences for the interaction between species of nematodes and initial population inoculated (Table 5).

The lowest TRS (Total Reducing Sugars), Brix% (total soluble solids) and PC (percentage of pol found



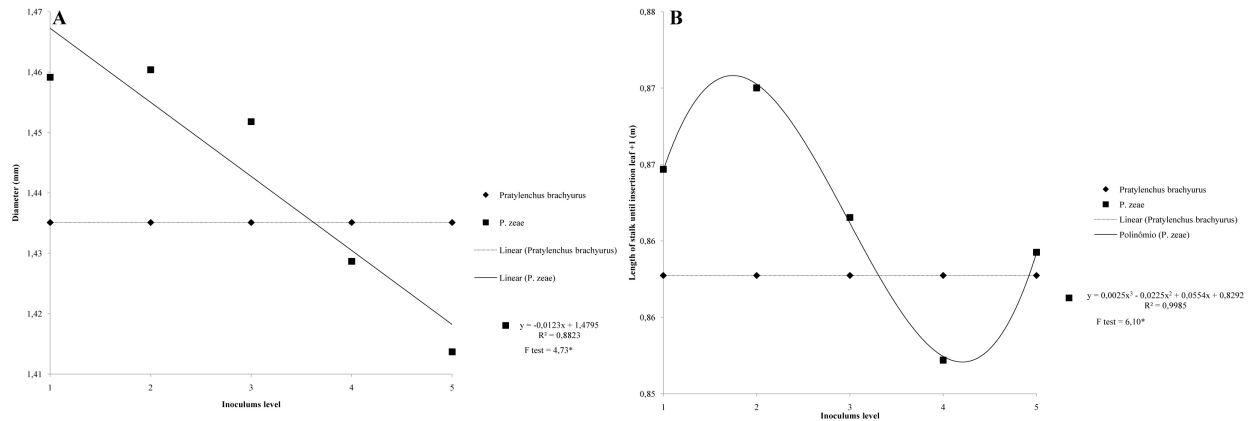


Fig. 5. Comparative effect of inoculation in increasing levels of *Pratylenchus brachyurus* (PB) and *P. zaei* (PZ), represented by black and red, respectively, on biometric variables. A) Diameter, B) length, C) Number of internodes and D) Estimated volume of the stalks of cane sugar variety CTC 2. The X axis with initial inoculum transformed into  $\log$ . Averages transformed into  $\log(x + 5)$  for regression analysis. Pradópolis, SP, October 20, 2008.

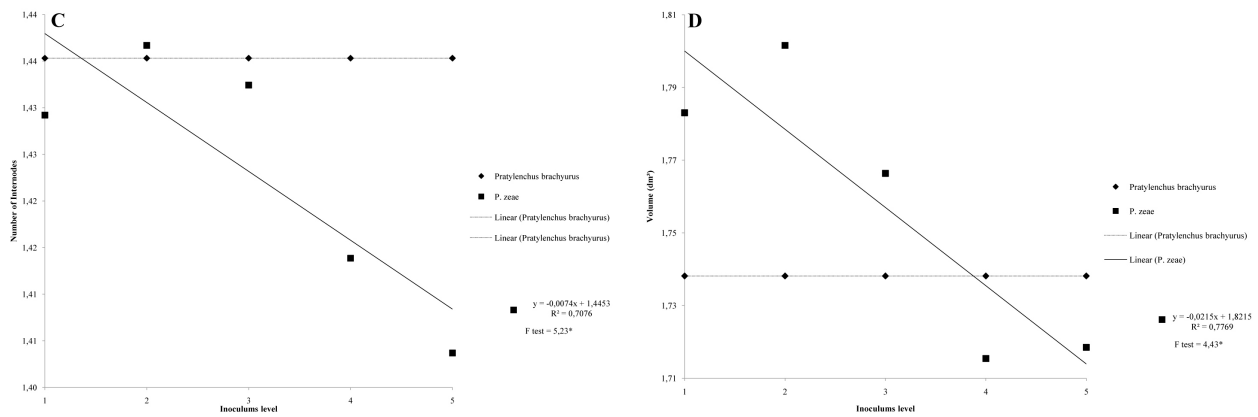


Fig. 5 (continuation). Comparative effect of inoculation in increasing levels of *Pratylenchus brachyurus* (PB) and *P. zaei* (PZ), represented by black and red, respectively, on biometric variables. A) Diameter, B) length, C) Number of internodes and D) Estimated volume of the stalks of sugarcane variety CTC 2. The X axis with initial inoculum transformed into  $\log$ . Averages transformed into  $\log(x + 5)$  for regression analysis. Pradópolis, SP, October 20, 2008.

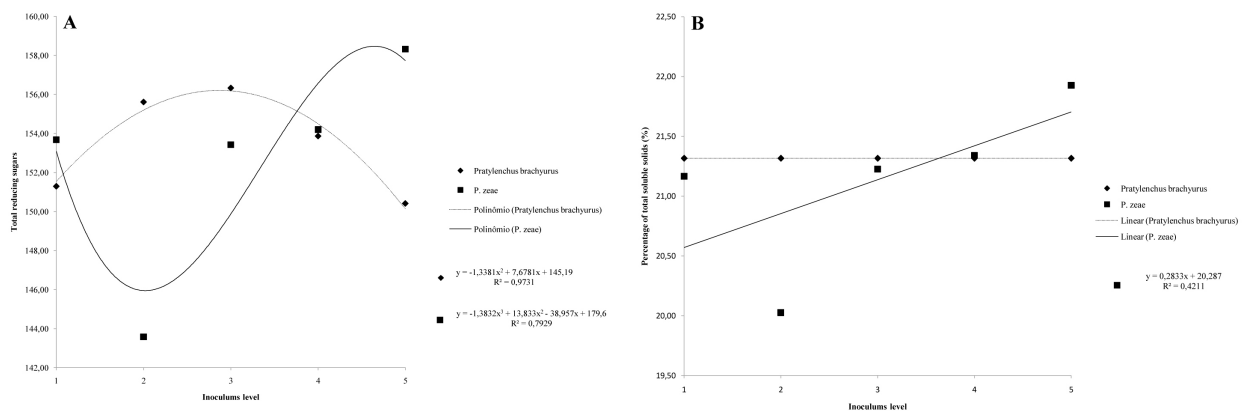


Fig. 6. Comparative effect of inoculation in increasing levels of *Pratylenchus brachyurus* (PB) and *P. zaei* (PZ), represented by black and red, respectively, on the variables A) total reducing sugars (TRS) and B) Percentage of total soluble solids (Brix%) of juice extracted from stalks of sugarcane variety CTC 2. The X axis with initial inoculum transformed into  $\log$ . Averages transformed into  $\log(x + 5)$  for regression analysis. Pradópolis, October 20, 2008.

## RESEARCH/INVESTIGACIÓN

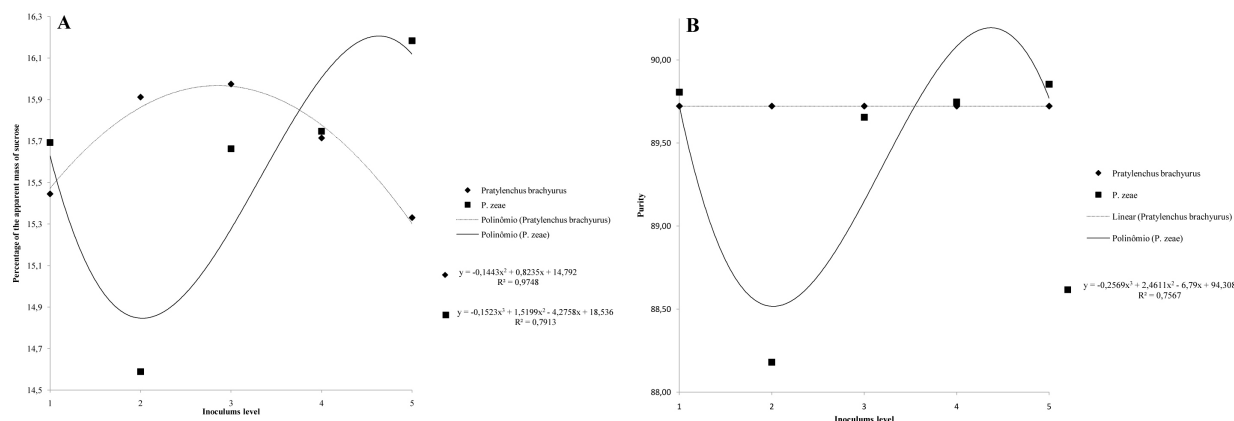


Fig. 7. Comparative effect of inoculation in increasing levels of *Pratylenchus brachyurus* (PB) and *P. zaeae* (PZ), represented by black and red, respectively, on the technological variables A) Percentage of the apparent mass of sucrose (PC) and B) purity or percentage of sucrose in relation to total soluble solids of the juice extracted from stalks of sugarcane variety CTC 2. The X axis with initial inoculum transformed into log. Averages transformed into  $\log(x + 5)$  for regression analysis. Pradópolis, October 20, 2008.



Figure 8. Visual aspect of treatments at harvest of sugarcane variety CTC 2. Control (T0), *Pratylenchus brachyurus* (N1) and *P. zaeae* (N2) inoculated with 10,000 specimens/plant (T4) were represented by T0, N1T4 and N2T4, left to right, respectively. October 20, 2008.

in sugarcane) were obtained for the initial inoculum level of 100 specimens/plant for *P. zaeae*, both when considering all levels of inoculum and in relation to *P. brachyurus* (Figures 6, 7 and 8). This fact is related to the small population that occurred at this same level of *P. zaeae* in the roots, when considering the average of all periods of evaluation (Fig. 4A). The highest value for the variables TRS, Brix% and PC was obtained for *P. zaeae* at the 100,000 specimens level and, again, the variable followed the population of nematodes for the level in question (Figs. 4A; 5A, B; 7A).

The values for Purity obtained for the plants that were inoculated with *P. brachyurus* did not differ (Fig. 7B). The 100 specimens/plant level of *P. zaeae* inoculum provided the lowest value both among *P. zaeae* and *P. brachyurus*. This is related to the low population numbers of *P. zaeae* in the roots for this level, when

considering the average of all assessment periods (Fig. 4A).

The values of TRS, PC and Purity in relation to the plants inoculated with increasing levels of *P. zaeae* were similar to the fluctuation of nematodes on the roots, for most evaluation periods (Fig. 4A). This trend also occurred in relation to TRS and PC, for the population of *P. brachyurus* in the soil and roots 120 days after inoculation of nematodes (Figs. 1A; 2A). The curves for variables TRS and PC in plants inoculated with *P. zaeae* are inversely proportional to that obtained for the length until leaf +1, and also have an inverse relationship to the diameter, number of internodes and stalk volume variables (Figs. 5; 5A; 7A).

The inoculum levels of *P. brachyurus* did not significantly affect the variables Brix% and Purity. This fact must be related to hypersensitivity or intolerance of

the variety CTC 2 to this nematode, since ten specimens of initial inoculum caused similar damage to that of 10,000 specimens of *P. zae* (Fig. 5A, 5D). There was an increase in Brix% values directly proportional to the increase in the level of initial inoculum of *P. zae*, but inversely proportional to the diameter, number of internodes and stalk volume, and with the opposite trend curve relative to the length of the leaf +1 (Figs. 4; 5B).

The highest values for the technological variables Brix%, TRS, PC and Purity, in relation to levels of inoculum of both species of nematode, were those relating to the stalks of plants inoculated with the level of 100 specimens of *P. brachyurus*/plant. However, the level of 100,000 specimens of *P. zae* provided higher values of TRS, PC and Brix% (Figs. 6 e 7). These results are similar to the population of nematodes for these two levels of each nematode, when considering the average population of all evaluation periods (Figs. 1A; 2A; 4A).

Plant parasitic nematodes cause a reduction in absorption capacity of water and nutrients. The plant is underdeveloped with smaller and fewer stalks (Table 4). Therefore, the greater the damage caused by nematodes, the higher the concentration of soluble solids present in the sugarcane stalk, including sugar, giving higher values of TRS, PC and Purity of the juice extracted from stalks (Figs. 5; 7). However, this does not yield benefits to the producer of sugarcane, because this increase in the TRS, PC and Purity is accompanied by low productivity.

Considering all facts *P. brachyurus* is more aggressive than *P. zae* to the variety of sugarcane CTC 2 because low initial population of *P. brachyurus* (10 specimens) was sufficient to cause similar damage to 10,000 specimens of *P. zae* (Figs. 5A, C, D; 8). Therefore, *P. brachyurus* should be included as key nematode in crops in Brazil.

#### ACKNOWLEDGEMENTS

The authors are grateful for the input of many colleagues, and especially to the São Martinho Plant, Pradópolis, SP, for their support. Special thanks to Mr. Marcos Marcari and the São Martinho Department of Agricultural Quality team, and Mr. André Ichinose, who lent the agricultural boiler used in experiment.

#### LITERATURE CITED

- Almeida, E. J. de, G. C. S. Alves, J. M. dos. Santos, and A. R. Ruas. 2011. Patogenicidade de *Meloidogyne mayaguensis* em goiabeira 'Paluma' em condições de microparcelas. Revista Brasileira de Fruticultura, 33:774-783.
- Barbosa, J. C.; W. Maldonado Jr. 2011. AgroEstat - Sistema para Análises Estatísticas de Ensaios Agronômicos, Version 1.1.0.668, Jaboticabal, São Paulo, Brazil.
- Barbosa, B. F., Santos, J. M. 2009. Study of the host-pathogen relationships of *Meloidogyne incognita* (Kofoid & White) Chitwood, *M. javanica* (Treub) Chitwood and *Pratylenchus brachyurus* (Godfrey) Filipjev & Schuurmans Stekhoven in sugar cane. Nematologia Brasileira, Piracicaba 33:304-305.
- Cadet, P., and V. W. Spaul. 2005. Nematode parasites of sugarcane. Pp. 645- 674 in M. Luc, R. A. Sikora, and J. Bridge, ed. Plant parasitic nematodes in subtropical and tropical agriculture. C.A.B. Wallingford: International Institute of Parasitology.
- Castillo, P., and N. Vovlas. 2007. *Pratylenchus* (Nematoda: Pratylenchidae): Diagnosis, Biology, Pathogenicity and Management Pp. 305-324 in P. Castillo e N. Vovlas, ed. Biology and Ecology of *Pratylenchus* vol. 6.
- Coolen, W. A., and C. J. D'Herde. 1972. A method for the quantitative extraction of nematodes from plant tissue. Ghent: Nematology and Entomology Research Station. 77 p.
- Dinardo-Miranda, L. L. 1990. Patogenicidade de *Pratylenchus brachyurus* e *Pratylenchus zae* (Nemata, Pratylenchidae) a duas variedades de cana-de-açúcar (*Saccharum* sp.). M.S. dissertation, College of Agriculture Luiz de Queiroz, Universidade de São Paulo, Piracicaba.
- Dinardo-Miranda, L. L. 2005. Nematoides e pragas de solo em cana-de-açúcar. Encarte do informações agronômicas 110:25-32.
- Dinardo-Miranda, L. L., C. C. Menegatti, V. Garcia, S. F. Silva, and M. Odorisi. 1998. Reação de variedades de cana-de-açúcar a *Pratylenchus zae*. STAB - Açúcar, Alcool e Subprodutos 17:39-41.
- Evans, K., and P. P. J. Haydock. 1990. A review of tolerance by potato plants of cyst-nematode attack, if consideration of what factors may confer tolerance and methods of assaying and improving it in crops. Annals of Applied Biology 117:703-740.
- Gonzaga, V. 2008. Caracterização morfológica, morfométrica e multiplicação *in vitro* das seis espécies mais comuns de *Pratylenchus* Filipjev, 1936 que ocorrem no Brasil. Ph.D. thesis, São Paulo Estate University, Faculty of Agricultural and Veterinary Sciences, Jaboticabal.
- Jenkins, W. R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Disease Reporter 48:692.
- Kimenju, J. W., S. W. Waudo, A. W. Mwang'ombe, R. A. Sikora, and R. P. Schuste. 1998. Distribution of lesion nematodes associated with maize in Kenya and susceptibility of maize cultivars to *Pratylenchus zae*. African Crop Science Journal 6:367-375.
- Landell, M. G. A., and M. A. Silva. 1995. Manual do experimentador – melhoramento da cana-de-açúcar. Pp. 3-9 in Metodologia de experimentação: ensaios de competição em cana-de-açúcar,

- Instituto agronômico, Pindorama.
- Hartman, K. M and J. N. Sasser. 1985 Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. Pp. 69-77 in K. R. Barker, C. C. Carter and J. N. Sasser, ed. Advanced Treatise on *Meloidogyne*, vol. II., Methodology. Raleigh: North Carolina State University.
- Southey, J. F. 1970. Laboratory methods for work with plant and soil nematodes. London: Minist. Agric. Fisch. Fd. Technical Bulletin No. 2. 148 p.
- Spaull, V. W., and P. Cadet. 1990. Nematode parasites of sugarcane. Pp. 461- 491 in M. Luc, R. A. Sikora and J. Bridge, ed. Plant Parasitic Nematodes in Subtropical and Tropical Agriculture. Paris: C.A.B. International Institute of Parasitology.
- Trudgill, D. L. 1991. Resistance to and tolerance of plant parasitic nematodes in plants. Annual Review of Phytopathology 29:167-192.

---

*Received:*

1/VIII/2012

*Accepted for publication:*

18/VI/2013

*Recibido:*

*Aceptado para publicación:*